

Parasitic Diatoms Inside Antarctic Sponges

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*Antarctic sponges may host large populations of planktonic and benthic diatoms. After settling on the sponge, these diatoms enter its body through pinacocytes (1) and form, there, large mono- or pauci-specific assemblages. Yet the total amount of carbohydrates in the invaded sponge tissue is inversely correlated with that of chlorophyll-*a*. We suggest, therefore, that endobiont diatoms utilize the products of the metabolism of their host as an energy source. This is the first evidence indicating that an endobiotic autotrophic organism may parasitize its animal host. Moreover, this unusual symbiotic behavior could be a successful strategy that allows the diatom to survive in darkness.*

Heterotrophic bacteria, autotrophic cyanobacteria, zoochlorellae, and zooxanthellae are common symbionts in Porifera, where they may actually constitute most of the sponge tissue. Sponges can also harbor fungal populations, the significance of which has been poorly investigated (2). The association of sponges with autotrophic symbionts occurs mainly in tropical (3) and temperate waters (4); it is a fruitful strategy, allowing the sponges to utilize the symbionts as a food source complementary to filter feeding (5, 6).

Diatoms in sponge tissues have rarely been recorded (7), but such associations are widespread in Antarctic waters (1). In fact, most sponges from Terra Nova Bay (Ross Sea, Antarctica) host large populations of both planktonic and

benthic diatoms, and these microorganisms are actively taken up by the pinacoderm and thus enter the sponge choanosome (1). This could be an adaptive supplementation of the food supply in the Antarctic ecosystem, because diatoms produce extracellular polysaccharides (8, 9) that sponges might use as energy substrates. On the other hand, the integrity of the diatom shells, the presence of cytoplasm inside the shells, and the formation of large mono- or pauci-specific assemblages inside a single sponge suggest that diatoms are able to complete their entire biological cycle inside the sponge body.

The aim of this study was to investigate the energy source that symbiotic diatoms might provide to Antarctic sponges. To this end, we evaluated the variation in the total carbohydrate content of sponges as a function of the biomass of endobiotic diatoms; biomass was estimated from chlorophyll-*a* concentration.

We examined 39 specimens representing 17 species of sponges (Table 1). The material was collected at Terra Nova Bay (Ross Sea), between 100 and 120 m depth, during the XIII Italian Antarctic Expedition (Austral Summer 1997–1998). The sponges were prepared for ultrastructural study as follows. Immediately after collection, small pieces from each specimen were fixed for 2 h in 2.5% glutaraldehyde in artificial seawater (ASW). The fixed tissues were rinsed, stored in ASW, and then dehydrated in a graded series of ethanol concentrations. Critical point drying was achieved with a CO₂ Pabish CPD 750 apparatus. Samples were examined with a Philips EM 515 scanning electromicroscope, and several species of diatoms were observed (Fig.

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Table 1

Chlorophyll-*a* concentration and carbohydrate content for the sponge specimens analyzed

Specimens	Chlorophyll- <i>a</i> ($\mu\text{g/g}$ dwaf)	Total sugars (mg/g dwaf)
<i>Artemisia tubulosa</i>	12.07 \pm 0.9	18.30 \pm 3.6
<i>Axociella nidificata</i>	27.29 \pm 2.5	8.12 \pm 0.2
<i>Dendrilla membranosa</i>	29.13 \pm 3.6	5.43 \pm 0.4
	30.47 \pm 2.1	11.40 \pm 2.1
	31.65 \pm 1.8	7.01 \pm 0.6
	21.34 \pm 1.9	16.29 \pm 2.8
	7.31 \pm 1	22.56 \pm 1.8
	9.96 \pm 0.9	27.06 \pm 1.4
	14.43 \pm 2.9	9.59 \pm 1.1
<i>Ectyodorix ramilobosa</i>	8.47 \pm 1.2	19.90 \pm 3.1
	20.70 \pm 3.1	6.91 \pm 1.8
<i>Gellius</i> sp.	17.05 \pm 2.4	7.25 \pm 2
<i>Gellius</i> sp. 1	37.10 \pm 6.9	6.91 \pm 1.5
<i>Haliclona dancoi</i>	16.67 \pm 2.4	9.92 \pm 2.4
	15.05 \pm 2.6	21.28 \pm 3.2
	15.14 \pm 0.9	8.10 \pm 1.4
	21.85 \pm 1.9	15.92 \pm 1.9
<i>Homaxinella balfourensis</i>	14.37 \pm 3.1	12.73 \pm 2.1
<i>Inflatella belli</i>	5.75 \pm 1.6	24.30 \pm 3.1
	2.21 \pm 0.8	24.41 \pm 3.6
<i>Isodictya conulosa</i>	16.48 \pm 1.6	12.29 \pm 2.2
	13.59 \pm 2	9.96 \pm 1
<i>Isodictya erinacea</i>	11.86 \pm 1.8	15.25 \pm 2.8
<i>Microxina benedeni</i>	11.75 \pm 3.2	11.21 \pm 1.2
<i>Phorbas glaberrima</i>	18.22 \pm 2.9	13.13 \pm 2.8
	19.04 \pm 4	12.73 \pm 4.1
<i>Pseudosuberites antarcticus</i>	44.56 \pm 3.5	3.32 \pm 0.9
<i>Pseudosuberites nudus</i>	27.79 \pm 5.6	10.23 \pm 1.4
	45.71 \pm 5.9	6.23 \pm 0.8
	9.13 \pm 1.7	17.71 \pm 2.5
	11.88 \pm 2.1	12.13 \pm 1.3
	24.53 \pm 4.8	9.23 \pm 1.9
	25.65 \pm 4.9	5.93 \pm 1.4
	19.74 \pm 3.6	7.41 \pm 2.8
<i>Suberites caminatus</i>	11.54 \pm 2.2	10.48 \pm 2.3
	32.94 \pm 3.8	8.91 \pm 1.7
<i>Tedania charcoti</i>	6.35 \pm 2.1	21.08 \pm 3.4
	17.25 \pm 5.7	7.11 \pm 1.5
	16.67 \pm 3.6	7.11 \pm 0.9

Values are the mean of three replicates \pm SD; dwaf, dry weight ash free.

l). Among pennate diatoms, *Fragilariopsis curta* (Fig. 1a), *Fragilariopsis* sp. (Fig. 1a, b), *Achnanthes* sp. (Fig. 1c), and *Pseudogomphonema* sp. were the most recurrent species; centric diatoms belonging to the genera *Porosira* (Fig. 1c), *Coscinodiscus*, and *Rhizosolenia* were rarer. For transmission electron microscopy, fragments of selected fixed material were postfixated in 1.0% OsO₄ in ASW at 4°C. After repeated rinsing, these fragments were dehydrated in ethanol and embedded in araldite. Ultrathin sections were cut with a Reichert ultramicrotome equipped with a diamond

blade; these sections were gathered on copper grids, stained with uranyl acetate and lead citrate for contrast, and examined with a Philips 300 transmission electron microscope. Micrographs (TEM) of *Fragilariopsis curta* provide evidence that the cytoplasm and thylacoids are intact (Fig. 1d).

The concentrations of chl-*a* in the various sponge specimens were measured and are listed in Table 1. These values differed widely, ranging from 2.2 $\mu\text{g/g}$ in *Inflatella belli*, to 45.7 $\mu\text{g/g}$ in *Pseudosuberites nudus*. High values were found also in *Pseudosuberites antarcticus*, *Gellius* sp. 1., *Suberites caminatus*, and *Dendrilla membranosa*. Strong differences emerged when different specimens of the same species were compared (e.g., from 7.3 to 31.6 $\mu\text{g/g}$ in *D. membranosa*; and from 9.1 to 45.7 $\mu\text{g/g}$ in *P. nudus*). This variation suggests that diatom uptake is fortuitous, depending primarily upon exposure of the sponge surface to diatoms settling upon it, rather than to some species-specific entrapping mechanism.

Carbohydrate in the sponge tissues (Table 1) ranged between 3.3 mg/g in *Pseudosuberites antarcticus* and 27.1 mg/g in *Dendrilla membranosa*. As with the chl-*a* concentration, carbohydrate content varied markedly when different specimens of the same species were compared.

For each specimen, we plotted carbohydrate content as a function of chl-*a* concentration and obtained the inverse correlation that is evident in Figure 2a. When we considered only those specimens belonging to a single species, this same trend was confirmed. In fact, in *Dendrilla membranosa* (Fig. 2b) and *Pseudosuberites nudus* (Fig. 2c), the total sugar content of the sponge tissue decreased as the concentration of chl-*a* increased.

The original aim of this work was to demonstrate that diatoms living inside the sponge tissue might represent a source of food for Antarctic sponges. This hypothesis seemed reasonable because diatoms produce large amounts of polysaccharides (8, 9). But our data show a clear inverse relationship between the amount of chl-*a* and of carbohydrate in sponge tissue. This relationship suggests that, as the population of diatoms within the sponge increases, it consumes more of the carbohydrate or related compounds produced by the sponge. This report, therefore, provides the first indication that diatoms in the field can utilize animal metabolic products as an energy source.

Like those in Antarctic sponges, most heterotrophic diatoms are pennate and occur in benthic habitats, where light is absent or limited and organic substrates are in great supply; such habitats strongly favor heterotrophic growth (10). Furthermore, several diatom species have developed active transport systems that bind to a limited number of organic substrates (glucose, lactate, and glutamate) and that respond to conditions of low light or darkness (11, 12); these diatoms have evolved highly sophisticated and ecologically significant mechanisms for

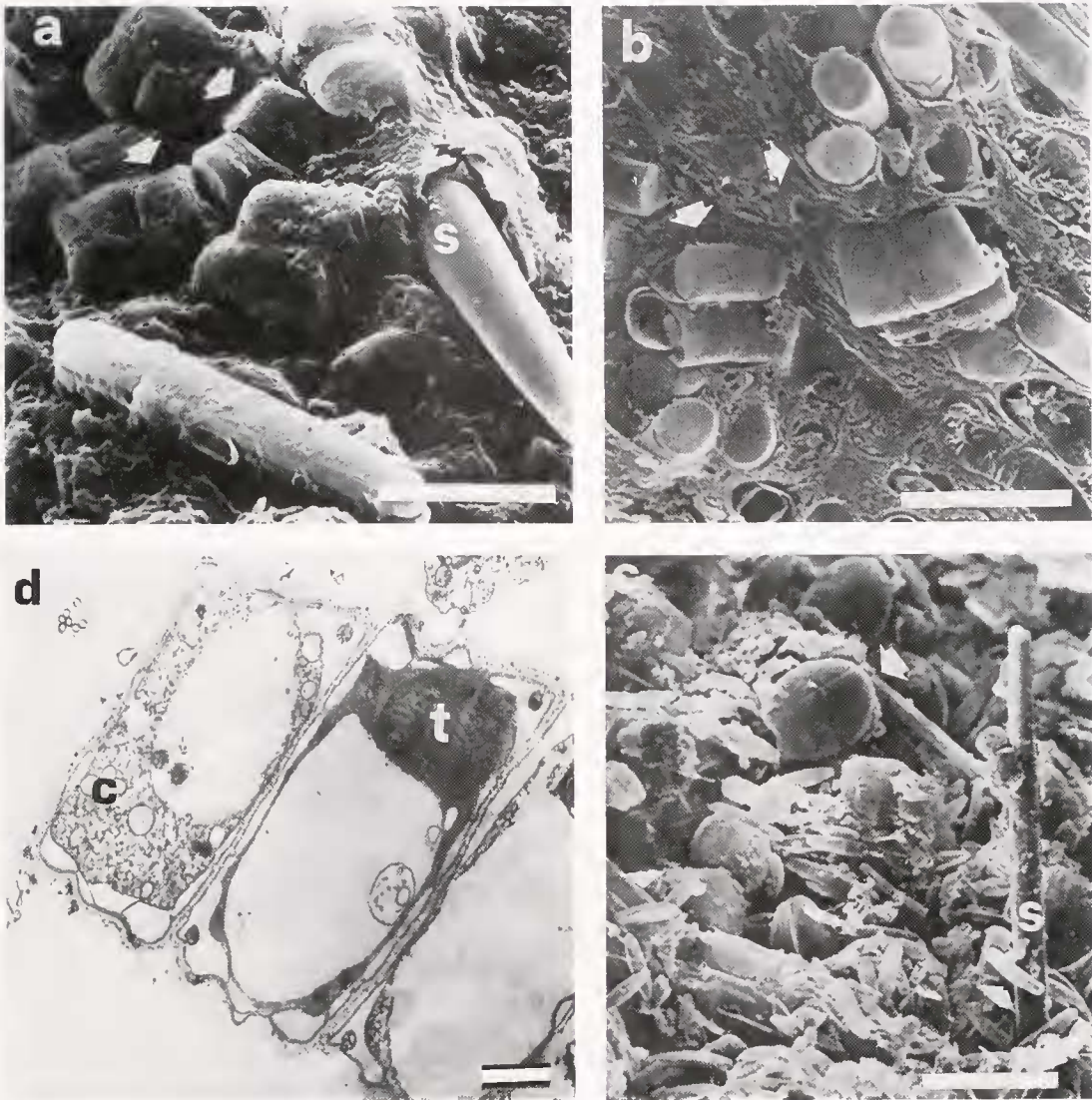


Figure 1. Diatoms living inside Antarctic sponges. (a) Several specimens of *Fragilariopsis curta* (arrows) and a *Fragilariopsis* sp. disposed under the exopinacoderm of *Tedania charcoti* (Scale bar, 50 μm). (b) *Fragilariopsis* sp. (arrows) embedded inside the same sponge (scale bar, 50 μm). (c) Large centric *Porosira* sp. (arrows) and numerous pennate *Aclmanthes* sp. (arrow heads) in *Pseudosuberites antarcticus* (scale bar, 100 μm). (d) Transmission electron micrograph of *Fragilariopsis curta* inside the sponge body showing intact cytoplasm (c) and thylacoid (t) (scale bar, 10 μm). s, sponge spicules.

facultative heterotrophy. Mixotrophy, a combination of autotrophy and heterotrophy, is widespread among certain protists and, in particular, among flagellates such as haptophytes (13), dinoflagellates (14, 15, 16, 17), cryptophytes (18), and diatoms (19, 20, 21). Previous documentation of mixotrophy in diatoms was obtained by observations *in vitro*.

The relationship between mixotrophic diatoms and Antarctic sponges can be considered as a sort of symbiosis shifting to parasitism; *i.e.*, the algae can get nourishment,

not only by photosynthesis, but also by utilizing organic carbon derived from their host. Although some Antarctic diatoms are considered among the most shade-adapted microalgae (23), the mixotrophy assumed in this report explains the surprising presence of dense diatom populations inside sponges living at 100–120 m depth, where the level of irradiance is very low (24).

Finally, the mechanism that causes a sponge to actively incorporate diatoms is unclear. However, some studies (22) have revealed a tendency of demosponges to incorporate

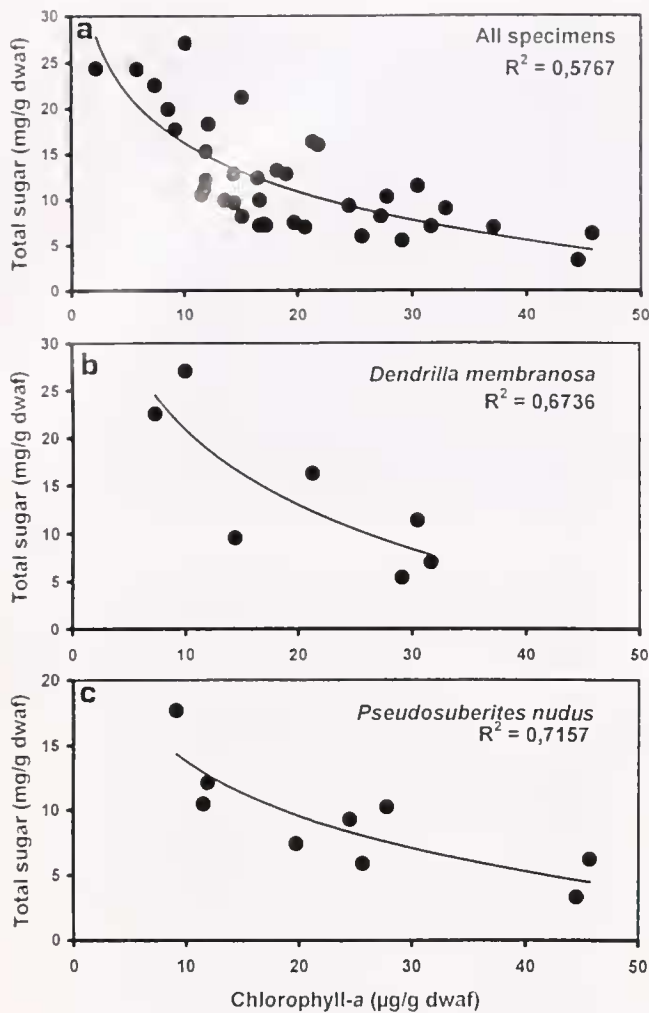


Figure 2. Concentration of chl-*a* as a function of the total amount of carbohydrate. Data from all the sponge specimens tested (a), from different specimens of *Dendrilla membranosa* (b), and from *Pseudosuberites nudus* (c). Each point represents a sponge specimen. The analyses were carried out on three samples of sponge tissue from each specimen. Samples were cylindrical cores of choanosomal tissue, 1 cm in diameter, 2 cm in height. Cores were longitudinally cut, and the two half-portions were tested to evaluate the chl-*a* concentration (as an estimate of diatom population biomass), as well as the total amount of carbohydrate. One subsample was extracted in 90% acetone. The chl-*a* was then assayed by spectrofluorimetry with excitation at 430 nm and emission measured at 668 nm. On the other subsample, the total carbohydrates were assayed: the method was based on the colorimetric reaction of sugars with phenol and sulphuric acid, and D(+) glucose was used as the standard (26).

siliceous particles, and the diatoms, with their siliceous shells, may thus "deceive" the sponge.

In conclusion, our data widen the ecological role of diatoms which, by exploiting the niche provided by sponges—one of the most important Antarctic benthic organisms—illustrate a strong functional link between plankton and benthos (25).

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